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Hydrothermal liquefaction of *Cyanidioschyzon merolae* and the influence of catalysts on products

Tapaswy Muppaneni [a,g], Harvind K. Reddy [a], Thinesh Selvaratnam [b], Kodanda Phani Raj Dandamudi [a], Barry Dungan [c], Nagamany Nirmalakhandan [d], Tanner Schaub [e], F. Omar Holguin [c], Wayne Voorhies [f], Peter Lammers [b], Shuguang Deng [a,g,*]

[a] *Chemical and Materials Engineering Department, New Mexico State University, Las Cruces, NM 88003, USA*
[b] *School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ 85287, USA*
[c] *Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003, USA*
[d] *Civil Engineering Department, New Mexico State University, Las Cruces, NM 88003, USA*
[e] *Chemical Analysis and Instrumentation Laboratory, New Mexico State University, Las Cruces, NM 88003, USA*
[f] *Molecular Biology, New Mexico State University, Las Cruces, NM 88003, USA*
[g] *School for Engineering of Matter, Transport and Energy, Arizona State University, Tempe, AZ 85287, USA*

HIGHLIGHTS

- Hydrothermal liquefaction of *Cyanidioschyzon merolae* and the influence of catalysts were investigated.
- Biocrude oil yield increased with temperature and with addition of catalysts.
- A correlation has been developed between the biochemical composition of algae and biocrude oil yield.
- Catalysts in HTL mixture improved the carbohydrate and soluble protein extraction in the water phase.

GRAPHICAL ABSTRACT

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ABSTRACT

This work investigates the hydrothermal liquefaction (HTL) of *Cyanidioschyzon merolae* algal species under various reaction temperatures and catalysts. Liquefaction of microalgae was performed with 10% solid loading for 30 min at temperatures of 180–300 °C to study the influences of two base and two acid catalysts on HTL product fractions. Maximum biocrude oil yield of 16.98% was obtained at 300 °C with no catalyst. The biocrude oil yield increased to 22.67% when KOH was introduced into the reaction mixture as a catalyst. The algal biocrude and biochar has a higher heating values (HHV) of 32.22 MJ kg^{-1} and 20.78 MJ kg^{-1} respectively when no catalyst was used. Gas chromatography time of flight mass spectrometry (GC/TOFMS) was employed to analyze the biocrude oil composition, and elemental analysis was performed on the algae, biocrude and biochar samples. Analysis of the HTL aqueous phase revealed the presence of valuable products.

1. Introduction

The current monetary development and the world's advancement have been, for the most part, determined by energy segment.

* Corresponding author at: Chemical Engineering Department, New Mexico State University, Las Cruces, NM 88003, USA.

E-mail address: shuguang.deng@asu.edu (S. Deng).

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The fast improvements of the world economy prompted the increment in the fossil fuel utilization, in this way expanding the fuel costs and creating ecological concerns. Accordingly, quickening the advancement and usage of new energy sources has turned into a typical activity around the globe. Biofuels can offer a conceivable course for the relief of carbon dioxide outflows caused by the fossil fuels and can possibly transform into another vitality source (Muppaneni et al., 2012; Yoo et al., 2015). Algal biofuels are third-generation biofuels that convert sunlight into synthetic energy through a green growth development, which can be utilized as raw materials for further biological or thermochemical transformation processes (Tian et al., 2014).

Algae can be grown in diverse water sources, for example, fresh water, salt water, and waste water and are extremely versatile to be grown under distinctive ecological conditions (Mata et al., 2010; Reddy et al., 2013; Selvaratnam et al., 2014). Algae basically comprises of lipids, proteins and sugars. Among these three macromolecules present in algae, numerous scientists are concentrating on converting over lipid parts into fluid powers, for example, biodiesel by means of transesterification. This is because of the way that algae can deliver higher oil yields contrasted with other customary oil seeds that are being utilized as feedstock for biodiesel production (Chisti, 2007). On the other hand, targeting on converting all the fractions of algae into biofuels yields higher biocrude oils that exceed the total lipid fraction present in the algae (Savage, 2012). Pyrolysis and Hydrothermal liquefaction (HTL) are some of the such processes that convert all the cellular components of algae into biocrude oil (Tian et al., 2014).

Pyrolysis requires dry biomass to be thermally deteriorated in the absence of oxygen to deliver bio-oil, biochar and uncondensable gas in which the bio-oil created is rich in oxygen content. Whereas, hydrothermal liquefaction converts algae with high moisture content into biocrude of higher heating value with less oxygen content compared to that of the oil from pyrolysis (Xu et al., 2014; Yang et al., 2014a). During HTL, the water at elevated temperatures and pressures acts as solvent, catalyst and as a hydrogen donor (Akiya and Savage, 2002). The oxygen present in the algal biomass will be removed by dehydration in the form of water and by decarboxylation in the form of carbon dioxide (Savage, 2009). The fragmentation of the macromolecules present in the algae will be done by hydrolysis and repolymerization relying on the process conditions (Akhtar and Amin, 2011; Toor et al., 2011). HTL yields four distinct products including biocrude oil, biochar, aqueous phase and gases. The biocrude oil was the main product and can be upgraded to be used as transportation fuel. The gaseous phase, for the most part, comprises of carbon dioxide and under 2% of hydrocarbon gasses (Eboibi et al., 2014). The nutrient rich (N, P) aqueous phase can be recycled to the algae cultivation ponds upon suitable dilution to be used as growth media for algae cultivation (Frank et al., 2013; Jena et al., 2011b; Selvaratnam et al., 2015a).

A few endeavors have been made by scientists to produce and to enhance biocrude oil yield from different types of algae using HTL strategy. Neveux et al., conducted HTL on fresh and marine water grown algal species and the biocrude oil yields were reported as 13.5% and 19.7% respectively (Neveux et al., 2014). In another study, Na_2CO_3 was used as a catalyst for the liquefaction of *Microcystis viridis* and achieved 33–40 wt% of biocrude oil yield (Yang et al., 2004). Minowa et al., reported 37% of biocrude oil yield when *Dunaliella tertrolecta* algae with ~20% lipids were liquefied using Na_2CO_3 as a catalyst (Minowa et al., 1995). Hydrothermal liquefaction of *Chlorella vulgaris* and *Spirulina* using alkali and organic acid catalysts was reported by Ross et al. (2010). Comparison of this literature for the production of biocrude oils is not evident as different algal species under different culture conditions and different biochemical compositions are used.

Several researchers have reported Na_2CO_3 as a catalyst for the liquefaction of biomass but very few researchers like Ross et al. (2010) reported HTL results using KOH, NaOH, CH_3COOH and H_2SO_4 as catalysts. Moreover, these homogeneous catalysts along with the water phase after HTL process can also be recycled to use it as growth media to grow *C. merolae* algal species. However, no work has been done on hydrothermal liquefaction of less known algal species *Cyanidioschyzon merolae*. This study mainly focusses on hydrothermal liquefaction of low lipid microalgae *Cyanidioschyzon merolae* and the analysis of product fractions. *C. merolae* is a unicellular red alga that was adapted to extreme thermo acidic conditions (pH 1.5, 45 °C) (De Luca et al., 1978). The influence of process temperature and the effect of acid and base catalysts on the biocrude oil yield was studied. Algae was analyzed for proximate and ultimate analysis. The biocrude oil produced was analyzed using gas chromatography/Time-of-flight mass spectroscopy (GC/TOFMS). The biocrude and biochar were analyzed for C, H, N, S content. The high heating values of algae, biocrude and biochar were determined using a bomb calorimeter. The aqueous phase has been analyzed by calorimetry.

2. Experimental

2.1. Materials

The microalgae used in this work *Cyanidioschyzon merolae* were grown outdoors in a standard cyanidium medium in a 4000-L horizontal photobioreactor at New Mexico State University algae cultivation facility. The chemicals, dichloromethane (DCM) and catalysts (KOH, NaOH, CH_3COOH and H_2SO_4) and all other solvents used in analysis were purchased from Sigma Aldrich, Saint Louis, MO, USA.

2.2. HTL experimental procedure

Hydrothermal liquefaction experiments were performed in a 100 mL bench top PARR 4593 Micro reactor with a 4843-controller (Parr Instrument Company, Illinois, USA) which can be operated up to 350 °C and 120 bars pressure. 50 g of microalgae slurry were prepared using harvested *C. merolae* and by adding sufficient supernatant water obtained during harvesting. This slurry was fed to the reactor and nitrogen was purged to sweep away the undesirable air in the reactor. The reactor was then heated after pressurizing with nitrogen to control rapid boiling of water and the residence time is taken from the point when the reactor reaches a desired temperature. All the experiments were conducted with 10% biomass loading, 30 min of reaction time and in triplicates by varying reaction temperatures from 180 °C to 300 °C. Catalytic HTL experiments were performed by adding different types of catalysts (KOH, NaOH, CH_3COOH and H_2SO_4) to make the reaction mixture approximately 0.5 M concentration.

After liquefaction, the reactor was cooled down to room temperature and the gases produced during the reaction were vented off. 30 mL of DCM was added to the reactor and stirred for 10 min to extract the biocrude oil present in the reaction mixture and from the reactor walls. The product mixture was then transferred to the separation unit to separate the biochar using a filter paper and to separate the DCM using a separation funnel. The biochar was washed with 5 mL of DCM to collect the residual biocrude and then dried before storing. The DCM was evaporated using rotary evaporator and thus separated biocrude oil along with the remaining aqueous phase were stored separately at −5 °C for further analysis. For non-catalytic HTL studies, yields of biocrude oil, biochar, water soluble compounds and gaseous products were calculated using equations presented elsewhere (Reddy et al., 2016).

In the case of catalytic HTL studies, the remaining mass fraction after calculating biocrude oil and biochar were included in one category due to the presence of catalysts in the aqueous phase.

2.3. Analytical methods

The residue left after heating the algae sample at 600 °C for 24 h was considered as ash. Fatty acid methyl ester (FAME) analysis was performed to determine the lipid content present in the microalgae and NREL protocol (Laurens, 2013) was followed to determine the total lipids present in the microalgae. The protein content was calculated as 6.25 times the amount of nitrogen evolved during the combustion of microalgae (Reddy et al., 2013). GC/TOFMS analysis of biocrude samples was performed for 90 min per sample using a modified Petroleum refinery reformate standard procedure (Corporation, 2010). Agilent 7890 A GC equipped with a ZB-5 ms column (30 m × 0.25 mm I.D. × .25 µm film thickness) with 1uL injections were made split less. The oven program started at 40 °C and held for 4 min then ramped at 5 °C/min to 110 °C, then ramped to 320 °C at 3 c/min. A 6725 Semi micro calorie meter manufactured by the Parr Instrument Company (Moline, Illinois, USA) was used to determine the high heating value of the microalgae, biochar and bio-crude oil samples. Ultimate Analysis of the dry biochar and biocrude samples was performed using an Elemental Analyzer (PE 2400 Series II CHNS/O Analyzer). Ammoniacal nitrogen (NH_3-N), Total nitrogen (TN), and phosphate in HTL aqueous phase (AP) samples were measured using HACH DR6000 Spectrophotometer (HACH, Colorado, USA) (Salicylate TNT Method 10031, TNT plus 828 Method 10208 and Phosver 3 Method 8048). Carbohydrate levels in the AP samples were measured using phenol sulphuric acid assay method (Dubois et al., 1956). All the HTL experiments and analysis are done in 3–5 replicates and the average results are reported.

3. Results and discussion

3.1. Characterization of C. merolae

The ash content in the *C. merolae* was 7.1% and moisture content was 67.13% after harvesting. This microalgae sample has a very low lipid content (1.99%) and high protein (56.5%) in it. Elemental analysis of this algae shows C, H, N, S content as 48.13, 5.14, 9.04 and 1.24% respectively. The ultimate analysis indicated that *C. merolae* has high oxygen content of 36.45%. The high heating value of this microalgae was 18.11 MJ kg^{-1} and was determined using a bomb calorimeter.

3.2. Effect of operating conditions on biocrude oil yield

All the hydrothermal liquefaction experiments were conducted at 30 min of reaction time, 10% of biomass loading and the effect of reaction temperature on the biocrude oil yield was investigated from 180 °C to 300 °C. The product yields obtained during the hydrothermal liquefaction of *C. merolae* are shown in Fig. 1. The biocrude oil yields are very low at temperatures below 225 °C. The yields were 0.65% at 180 °C and 2% at 200 °C. These lower yields are due to the lower lipid content in the microalgae and the inability of water to hydrolyze other components up to 200 °C. Above 225 °C, the hydrolysis became predominant causing more yields at higher temperatures. At 225 °C the biocrude oil yield obtained was 4.95% which is well above the amount of lipids present in the microalgae. The biocrude oil yields increased to 11% at 250 °C which shows rapid formation of biocrude oil from 200 °C to 250 °C. Further increase in the temperature increased the biocrude oil yield. A biocrude oil yield of 15.74% was obtained at 275 °C and a yield of 16.98% was obtained at 300 °C. It is evident that the increase in the temperature increases the biocrude oil yield due to the formation of biocrude oils not only from lipids but from other non-lipid components (proteins and carbohydrates) as well. The high temperatures encourage the cleavage of bonds like peptide bonds, C-C bonds and C-O-C bonds to fragment the basic compounds and enhanced reactions like hydrolysis and repolymerization which improves the bio-oil yield at various process conditions (Akhtar and Amin, 2011; Toor et al., 2011).

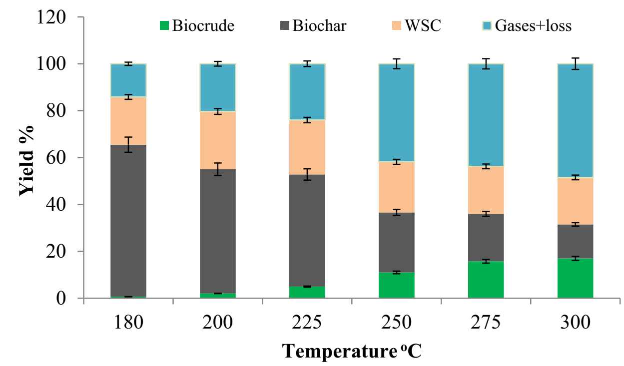

Fig. 1. HTL product distribution of *C. merolae.*

Further increase in temperatures might lower the yields due to intensified gasification at higher temperatures (Brown et al., 2010; Reddy et al., 2016). Reddy et al., reported the decrease in the biocrude oil from 47.5% to around 40% when the temperature was increased from 300 °C to 330 °C with Nannochloropsis sp. (Reddy et al., 2016). The biocrude oil yields obtained in this study are based on dry weight and were similar to that of the biocrude oils produced from *Chlorella sorokiniana* microalgae (18%) (Reddy et al., 2013) and from macro algae *Laminaria saccharina* (19.3%) (Anastasakis and Ross, 2011) which were determined on the basis of ash free dry weight (AFDW). Even though the biocrude oil comprises of components fragmented from lipids, carbohydrates and proteins, the higher lipid content in the algae can result in the higher biocrude oil yield (Neveux et al., 2014). A correlation between biochemical composition (lipids, proteins and carbohydrates) and biocrude oil yield has been developed using our HTL results from this study and our previous studies (Reddy et al., 2013, 2016), a simple formula created based on measured biocrude oil yield results is as follows

$$\begin{aligned}\text{Biocrude oil yield }\% = {} & (130.6 * L) + (37.02 * P) - (74.45 * C) \\ & - (1.096 * L * L) - (0.4386 * P * P) \\ & + (0.8554 * C * C) - (1.887 * L * P) \\ & - (0.8371 * L * C) + (0.4563 * P * C)\end{aligned}$$

where L is lipids, P is proteins and C is carbohydrates present in algae.

The biochar yields decreased with the increase in the temperature from 64.85% at 180 °C to 14.50% at 300 °C which is consistent with our previous studies (Reddy et al., 2016), but not consistent with the work by Zhou et al., who reported the biochar yields decreased from 20.2% at 220 °C to 16.9% at 320 °C where there is less than 5% decrease in the biochar yield with 100 °C raise in the HTL temperature (Zhou et al., 2010). The decrease in the biochar yield with increasing temperature is due to the conversion of the constituent components to biocrude oil, water soluble compounds and gaseous products at higher temperatures, which are not converted at lower temperatures.

The water soluble compounds were 20.36% at 180 °C and increased to a maximum yield of 24.63% at 200 °C further increase in the temperature decreased the WSC yield gradually and lower WSC yield of 20.04% was obtained at 300 °C. This decrease in the WSC above 200 °C might be due to the repolymerization of water soluble compounds into biocrude or gaseous phase. Zhou et al. and Alba et al. also reported the conversion of water soluble products into biocrude oil at higher temperatures (Alba et al., 2012; Zhou et al., 2010). The gaseous products were very low at 180 °C and increased with the increase in temperature. The lowest gas yield was observed as 14.14% at 180 °C and increased to 24% at 225 °C. Further temperature raise from 225 °C to 250 °C doubled the biocrude oil yield and almost doubled the gaseous product yield and reduced the biochar yield to half. This might be the transition point which triggered the major conversion of biochar into biocrude and gaseous products. Further increase in the temperature increased the gaseous product yield and a maximum yield of 48.48% was obtained at 300 °C.

3.3. Effect of catalysts on biocrude oil yield

The introduction of catalyst into the HTL system is to increase the yield of biocrude and to improve fuel properties. Minowa et al. reported that the introduction of sodium carbonate into the HTL system increased the biocrude oil yield at 300 °C and decreased the oxygen content in the biocrude oil at 200 °C for Dunaliella tertiolecta algal species (Minowa et al., 1995). Whereas Anastasakis et al. reported that KOH as a catalyst in HTL mixture lowered the biocrude oil yield and increased the yield of water soluble compounds for *Laminaria saccharina* species (Anastasakis and Ross, 2011). Four catalysts (two acid and two base) were used in this study to investigate the effect of catalysts in the liquefaction and the product yields were shown in Fig. 2. The biocrude oil yields increased from 16.98% without catalyst to 21.23% with CH_3COOH, 21.78% with NaOH and to 22.67% with KOH and no noticeable increase in the biocrude oil yield was observed with H_2SO_4. Similar results were observed by Zhou et al., while using macroalgae *Enteromorpha prolifera* with catalysts where the maximum biocrude oil yields were 23% with Na_2CO_3 as a catalyst and 20% without the catalyst (Zhou et al., 2010).

Ross et al., found that the organic acids increases the biocrude oil yield and these organic acids will be consumed acting as a reagent rather than a catalyst (Ross et al., 2010). They also reported that the organic acid catalysts produce higher biocrude oil yields than that of alkali catalysts, but the biocrude oil yields in this study were similar for both the organic acid and alkali catalysts. As shown in Fig. 2, all the catalysts used in this study except H_2SO_4 tend to increase the biocrude oil yields but decreased the biochar yields. However, the acid catalyst H_2SO_4 showed no effect of biocrude oil yield but increased the biochar yield. Yang et al., studied the effect of sulfuric acid as a catalyst and observed increase in the

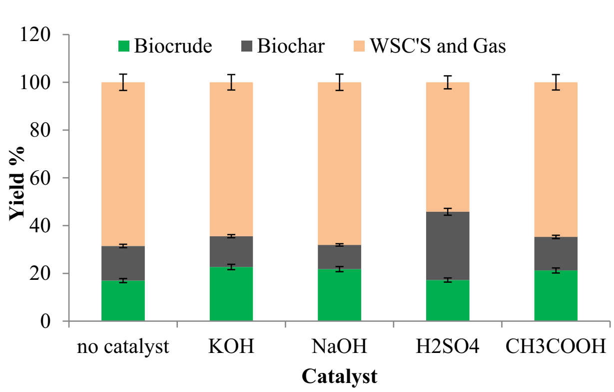

Fig. 2. HTL product distribution for *C. merolae* with catalysts.

biochar yield and decrease in the biocrude oil yield, this might be due to the biocrude oil tending to form biochar by polymerization under acid conditions (Yang et al., 2014b). Even though all the catalysts except sulfuric acid significantly increased the biocrude oil yield, KOH seems to be more effective because of the higher biocrude oil yield compared to other catalysts.

3.4. Analysis of liquefaction products

3.4.1. GC/TOFMS analysis of biocrude oil

The biocrude oil has been analyzed by a GC/TOFMS and the main compounds were identified by NIST library. The biocrude oil was a complex mixture and more than 1000 compounds were detected, and more than 600 compounds were identified by the library. The principle segments (with area % >1) detected in biocrude oil obtained during direct HTL without catalysts at 300 °C, 30 min of reaction time and 10% solids was presented in Table 1. The biocrude oil may contain different compounds like amides, amines pyrollidine, hexadeconoic acid, indole, phenol piperidine, ketones, alkanes, alkenes and furans (Jena et al., 2012). The protein content present in the algae was rapidly hydrolyzed to amino acids which in turn converted into amines and amides by various decarboxylation and deamination reactions (Ross et al., 2010). The pyrazine derivatives might be due to the maillard reactions which are reactions between amines and sugars. The identified ketones and phenols were produced from carbohydrates via hydrolysis, dehydration, cyclization etc and alkenes might be generated from the conversion of unsaturated fatty acids presenting in the algae sample (Zhou et al., 2010). The compounds detected in the biocrude oils produced from *C. merolae* were somewhat similar to the literatures but more compounds with less relative abundance (in% of a total area of GC peaks) were identified in this work. This might be due to the different GC/TOFMS procedure implemented for analysis of the biocrude oils and the chemical composition of the algal species.

3.4.2. Elemental analysis and HHVs of biocrude oil and biochar

The elemental compositions of biocrude oils and biochar obtained at 300 °C with and without a catalysts are shown in Table 2. The carbon content in the biocrude oil obtained without a catalyst was 74.53% and was higher than the carbon content in

Table 1
Main compounds detected in the biocrude oil obtained without catalyst at 300 °C, 30 min and 10% solids by GC/TOFMS.

Name	Retention time (min)	Relative abundance (area %)
TrimethylPyrazine	11.49	1.19
4-methylPhenol	14.53	1.09
1-(2-Isopropenyl-5-methylcyclopentyl) ethanone	43.56	1.15
11-Hexadecyn-1-ol	43.57	1.26
5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a;1′,2′-d]pyrazine	46.37	2.97
2-(*tert*-Butylamino)ethyl methacrylate	46.93	1.37
Dodecanamide	54.30	3.35
N-Methyldodecanamide	55.20	2.47
N,N-Dimethyldodecanamide	56.45	1.74
N-(n-Propyl)acetamide	59.16	1.30
Tetradecanamide	60.30	2.01
3-Benzylhexahydropyrrolo[1,2-a] pyrazine-1,4-dione	60.33	1.04
N,N-Diethyldodecanamide	60.56	1.43
N-Acetylethylenediamine	60.58	1.25
N-Methyldodecanamide	61.18	1.19
1-Hexadecanamine	61.19	1.19
N-Decanoylmorpholine	63.43	2.03

Table 2
Elemental composition of biocrude oils and biochar obtained at 300 °C.

Biocrude oil	C	H	N	S	O[a]	HHV
No catalyst	74.53	4.65	7.37	1.04	12.41	32.22
KOH	76.80	5.11	5.74	0.86	11.49	33.66
NaOH	74.03	6.97	5.07	1.02	12.91	32.89
H_2SO_4	70.75	4.0	3.72	2.47	19.06	33.76
CH_3COOH	74.0	8.37	6.26	2.10	9.27	33.36
Biochar						
No catalyst	56.86	5.66	5.57	1.03	30.88	20.70
KOH	49.30	4.91	3.47	1.68	40.64	14.64
NaOH	54.01	4.79	4.70	2.20	34.3	11.74
H_2SO_4	43.52	4.85	3.36	5.49	42.78	14.69
CH_3COOH	50.19	4.66	4.12	3.24	37.79	12.25

HHV = higher heating value (MJ kg^{-1}).
[a] Determined by difference.

the algae feedstock which was 48.13%. The carbon content present in the biocrude oil obtained with catalysts was similar to that of the carbon content in biocrude oil without a catalyst. The introduction of catalysts in the HTL improved the hydrogen content in the biocrude oils. The nitrogen content in the biocrude oil was lower than the fresh algae which might be due to the formation of nitrogen based compounds into the aqueous phase. The nitrogen content was reduced during the HTL compared to the fresh algae and further reduced when catalysts were added in the reaction mixture. Oxygen contents in the biocrude oils were decreased significantly to 12.41% without catalyst compared to 36.45% in the fresh *C. merolae*. The catalyst showed no significant effect on the oxygen content except for the acetic acid where the oxygen content was reduced to 9.27%. Even though the oxygen content and nitrogen content were reduced in the biocrude oil, further upgrading of oils is required through deoxygenation and denitrogenation to avoid unwanted gases like NOx during combustion.

The biochar has a carbon content similar to that of fresh algae when non catalytic HTL was implemented while the carbon content was decreased with the catalytic influence. This decrease might be due to the formation of carbon based gases with the introduction of catalysts. The higher heating value of the biocrude oil was 32.22 MJ kg^{-1} and is higher than the HHV of algae which was 18.11 MJ kg^{-1}. Similar HHVs were obtained by Zhou et al. (28–30 MJ kg^{-1}) using *E. prolifera* and the higher HHVs (34.7–39.9 MJ kg^{-1}) was obtained by using *S. plantesis* algal species (Jena et al., 2011a; Zhou et al., 2010). The catalysts did not significantly influence the HHV of biocrude oil and the maximum HHV of 33.76 MJ kg^{-1} was obtained when sulfuric acid was used as a catalyst. The higher energy content of the biochar might be due to the residual carbohydrates present in the biochar (Williams and Laurens, 2010). According to Toor et al., the crude proteins will be preserved in the biochar and has great nutritional value, the biochar produced in the HTL process can be used as an animal feed additive (Toor et al., 2011).

3.4.3. Analysis of HTL aqueous phase

The HTL aqueous phase obtained after a typical HTL run consists of nutrients and valuable co products. Nutrients like NO_3^-, PO_4^{3-}, K and NH_4^+ were reported to be present in the residual aqueous phase after the hydrothermal liquefaction of algal biomass (Biller et al., 2012; Jena et al., 2011b). The aqueous phase was analyzed for the presence of nutrients like ammoniacal nitrogen, total nitrogen and phosphate levels. The presence of valuable co products such as carbohydrates and soluble protein were also analyzed in this study. The aqueous phase analysis of *C. merolae* without catalysts was presented in Table 3. Ammoniacal nitrogen (NH_3-N) was detected in the HTL aqueous phase and the presence of ammoniacal nitrogen increased with the increase in the reaction temperature. The total nitrogen also increased with increase in temperature till 250 °C and further increase in temperature showed little effect on the total nitrogen extraction. The proteins present in the algae might decompose and extracted into aqueous phase as amino acids and ammonia due to the degradation of algal biomass (Biller and Ross, 2011; Valdez et al., 2012). But at 300 °C both the total and ammoniacal nitrogen levels tend to decrease compared to the nitrogen levels obtained at 275 °C. This might be due to the conversion of those amino acids into biocrude oil at higher temperatures via deamination and decarboxylation (Moscoso et al., 2013).

The phosphate levels (239 mg L^{-1}) was observed to be maximum at 180 °C and decreased to 22 mg L^{-1} with the increase in the temperature to 300 °C. Co products like sugars and soluble protein can be extracted into aqueous phase during HTL. These extracted coproducts are very valuable and have many commercial applications. Carbohydrate levels of 21,395 mg L^{-1} was observed at 180 °C and increase in the temperature lowered the carbohydrate extracts to 200 mg L^{-1} at 300 °C as they were further converted into biocrude oil at higher temperatures. The soluble proteins also showed the same trend as carbohydrates. The soluble proteins extracted at 180 °C were 1024 mg L^{-1} and then decreased to 344 mg L^{-1} at 300 °C. Due to the presence of carbohydrates, proteins, nutrients and phosphates in the HTL aqueous phase; this by-product from the HTL process can be used to grow algae under mixotrophic conditions necessary dilutions (Selvaratnam et al., 2015b).

Table 4 shows the analysis of water soluble compounds present in the HTL aqueous phase when catalysts were used. The introduction of catalysts decreased the ammoniacal nitrogen and total

Table 3
HTL AP analysis of *C. merolae* without catalysts.

Temp °C	$N-NH_3$	TN	Phosphate [mg/L]	Carbohydrate	Soluble protein
180	830	5067	239	21395	1025
200	1593	6007	174	7004	450
225	2280	6370	37	1496	723
250	3280	7820	28	840	767
275	3890	8357	23	379	616
300	3833	6703	22	200	344

Table 4
HTL AP analysis of *C. merolae* with catalysts.

Catalyst	$N\text{-}NH_3$	TN	Phosphate [mg/L]	Carbohydrate	Soluble protein
No catalyst	3833	6703	22	200	344
NaOH	3000	4626	1	1238	1902
KOH	3366	4620	1	1140	1387
CH_3COOH	2826	5476	3	540	304
H_2SO_4	5743	7983	2	543	348

nitrogen levels except for the sulfuric acid. The ammoniacal nitrogen increased from less than 400 mg L^{-1} to around 5500 mg L^{-1} when sulfuric acid was used as a catalyst. Whereas the phosphate levels decreased from 22 mg L^{-1} to less than 5 mg L^{-1} when catalysts were added in the system. The catalysts increased the carbohydrate and soluble protein levels in the HTL aqueous phase. The acid catalysts showed little influence on the carbohydrate and soluble protein levels while the base catalysts showed a significant increase in the extraction of carbohydrates and soluble proteins into the aqueous phase.

4. Conclusions

This study evaluated the influence of catalysts on hydrothermal liquefaction of *Cyanidioschyzon merolae*. The biocrude oil increased with temperature and maximum biocrude of 16.98% was obtained at 300 °C with no catalyst. More biocrude oil was produced with KOH catalyst than other catalysts and a maximum biocrude yield of 22.67% was obtained at 300 °C, 30 min and 10% solid loading. The HHV of biocrude oil obtained with a catalyst was similar to that of the biocrude obtained without catalysts. The introduction of catalyst in the HTL process increased the carbohydrate extraction but decreased the phosphate recovery into the water phase.

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